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REMARKS

The Examiner is requested to consider the accompanying remarks.
Reconsideration of the present application is respectfully requested.

Claims 9-25, 28-31, 54-59, 61, 63-67, 69-73, 76-87, and 96-99 are in the application for consideration.

Claim 73 has been cancelled without prejudice.

The specification has been amended to address the Examiner's objections and comply with the sequence rules.

The marked-up version of these amendments is found on a separate sheet attached to this amendment and titled **"Version with Markings to Show Changes Made."** It is respectfully requested that the amendments be entered.

Objections to the specification

19. The Examiner states: "The specification is objected to for being confusing on page 8. As amended on January 31, 2001, the paragraph discussed "organisms" that are modified. However, the list that follows refers to SEQ ID NOs of sequences that are modified. Appropriate clarification is requested."

No amendment is recorded for January 31, 2001, so it is assumed that the amendment the Examiner is referring to is the amendment mailed November 28, 2001, which appears to contain the paragraph in question.

The paragraph on page 8 beginning on line 16 has been amended to delete the SEQ ID NOs. It is believed this clarifies the meaning of the specification.

20. The Examiner states: "The specification is objected to for being confusing with respect to the sequence listing. The sequence contains 74 sequences. Every SEQ ID NO is mentioned in the specification and/or the claims except SEQ ID NOs: 33-34.... All SEQ ID NOs in the sequence listing must be described in the specification. Appropriate correction or clarification is required."

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The specification has been amended on page 5, line 18 (Figure 2) to include SEQ ID NOS: 33 and 34 which have been annotated as consensus sequences.

Objections to the Claims

21. Claims 10, 13, 20, 23, 24, 56, 69, 71, 85, and 86 are objected to for having an improper Markush group.

The Examiner states: "The pairs of substitutions should be joined by an 'and' not an 'or' as claimed. Appropriate correction is required."

The Examiner's objection is respectfully traversed. Claims 10, 13, 20, 23, 24, 56, 69, 71, 85, and 86 are not written in Markush format. A Markush format utilizes the term "the group consisting of". The present claims are written in acceptable alternative format as exemplified in MPEP 2173.05(i)(II): "Alternative expressions using 'or' are acceptable, such as 'wherein R is A, B, C, or D.'"

22. Claim 73 is objected to under 37 U.S.C. §1.75 as being a substantial duplicate of Claim 64.

The Examiner is thanked for pointing out the duplication. Claim 73 has been cancelled.

23. Claims 11, 12, 14-18, and 96 are objected to for depending on objected claims.

The objection is traversed for the reasons stated above in reference to claims 10, 13, 20, 23, 24, 56, 69, 71, 85, and 86.

Claim Rejections - 35 U.S.C. §112

24. Claim 99 is rejected under 35 U.S.C. §132 and 35 U.S.C. §112, first paragraph, new matter, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art

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that the inventor(s), at the time the application was filed, had possession of the claimed invention.

The Examiner states: "The amendment filed on September 3, 2002, adds new matter to Claim 99 as follows: 'at least 15-25 mole % lysine'. Applicant is required to cancel the new matter in the reply to Office Action or to identify clear support (including page and line number) for the above phrase in the specification as originally filed."

Claim 99 is retained. Support for the amendment of September 3, 2002 is found in the specification on page 45, beginning on line 15 which states: "One embodiment of the present invention provides an isolated polypeptide comprising a plant substituted CI-2-like polypeptide having the following composition: 15-35 mole % *lysine*...." (italics inserted). As the claimed limitation of '15-25 mole % lysine' is encompassed by the disclosed range, there is clear support in the specification as filed for the amendment.

25. Claim 29 is rejected under 35 U.S.C. §112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

The Examiner states: "The term 'conservative substitution' is unclear as to its metes and bounds. Applicants amended the specification to include on 'conservatively substituted variants' which, they attest, are limited to the substitutions listed on page 10 of the specification. However, this citation in the specification is unclear.... The Examiner suggests substituting it with --conservatively substituted polypeptide thereof -- provided that the term 'conservatively substituted' can be made clear in light of the specification as originally filed."

Claim 29 has been amended to incorporate the Examiner's suggestion.

The paragraphs on page 10 listing amino acids that are conservative amino acid substitutions for each other, first list substitutions that are grouped by standard chemical characteristics, e.g.: acidity, hydrophobicity, etc. The second two groups

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are characterized by their ability to substitute also as essential amino acids in the claimed polypeptide.

Claim Rejections under 35 USC §102

26. Claims 98-99 are rejected under 35 U.S.C. §102(b) as being anticipated by Kleber-Janke *et al.*

The Examiner states: "Kleber-Janke *et al.* teach a barley polypeptide having 43% sequence identity with SEQ ID NO:2 and having 10 (out of 68 residues) lysine residues for a mole percent of 14.7%...."

The rejection is respectfully traversed. Present claims 98 and 99 read: "A polypeptide with at least 30% sequence identity to the polypeptide of Seq. ID No. 2 and comprising greater than fifty amino acids in length and *modified* in order to have a composition of *at least 15.00-35.00 [25] mole % lysine; wherein the % sequence identity is based on the entire sequence and is determined by BLAST 2.0 using default parameters.* (italics inserted).

The sequence in Kleber-Janke *et al.* was not modified; further, the mole percent lysine, by the Examiner's own calculations was not "at least 15%" but 14.7%; and last, the percent sequence identity was not based upon the entire length of SEQ ID NO:2 but on merely a portion of the sequence: from amino acids 21 through 83.

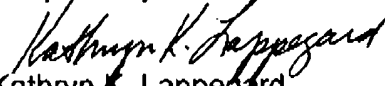
It is well established law that patentability is negated under 35 U.S.C. §102 only when the prior disclosure is identical to the invention sought to be patented. To anticipate a claim, a single source must contain all of the elements of the claim. See *Hybritech Inc. v. Monoclonal Antibodies, Inc.*, 802 F.2d 1367, 1379, 231 USPQ 81, 90 (Fed. Cir. 1986); *In re Marshall*, 578 F. 2d 301,304, 198 USPQ 344, 346, (CCPA 1978). Accordingly, it is asserted that Kleber-Janke *et al.* do not teach the polypeptide of claims 98 and 99 and request the Examiner withdraw this rejection.

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CONCLUSION

In view of the above amendments and remarks, it is submitted that the objections to the specification and to claims 10-18, 20, 23, 24, 56, 69, 71, 73, 85, 86, and 96 are overcome. It is respectfully submitted that the rejection of claims 99 and 29 under 35 U.S.C. §112, first and second paragraphs, are overcome as well as the rejection of claims 98 and 99 under 35 U.S.C. §102(b). Applicants respectfully request reconsideration of this application and its allowance.

Respectfully submitted,


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VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the claims:

Claim 73 has been cancelled without prejudice.

Claim 29 has been amended as follows:

29. (Four Times Amended) An isolated polypeptide comprising Seq. ID No. 6, 8, 10, 12, 14, 16, 18, 20 or a conservatively substituted polypeptide ~~conservative substitution~~ thereof, wherein said isolated polypeptide or conservatively substituted polypeptide ~~conservative substitution~~ thereof has at least 30% sequence identity to the polypeptide of Seq. ID No. 2, wherein the percent identity is determined by Blast 2.0 using default parameters

In the specification:

Paragraph beginning at line 16 of page 8 has been amended as follows:

A "CI-2 like" polypeptide refers to a polypeptide of at least 23 consecutive amino acids of Seq. ID No. 2 or 4; or a polypeptide of at least 30% amino acid sequence identity with corresponding region of Seq. ID Nos. 2 or 4 or 20; or a CI-2 like polypeptide with modifications identified in CI-2; or a protease inhibitor with an active site loop typically between 53 and 70; or a CI-2 homologue modified to enhance its nutritional value by altering the amino acid residues at positions corresponding to those defined herein. The following organisms (~~Genebank Accession Numbers~~) may be modified according to the methods and figures in the specification *Hordeum vulgare* (Seq. ID No. 35), *Hordeum vulgare* (Seq. ID No. 36), *Zea mays* [(Seq. ID No. 37)], *Vicia faba* (Seq. ID No. 38), *Cucurbita maxima* (Seq. ID

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~~No. 39), Canavalia lineata (Seq. ID No. 40), Vigna angularis (Seq. ID No. 41),
Nicotiana tabacum (Seq. ID No. 42), Nicotiana glauca (Seq. ID No. 43),
Sambucus nigra (Seq. ID No. 44), Momordica charantia (Seq. ID No. 45), Cucurbita
maxima (Seq. ID No. 46), Solanum tuberosum (Seq. ID No. 47), Solanum
tuberosum (Seq. ID No. 48), Lycopersicon peruvianum (Seq. ID No. 49),
Lycopersicon esculentum (Seq. ID No. 50), Lycopersicon esculentum (Seq. ID No.
51), Amaranthus caudatus (Seq. ID No. 52), Arabidopsis thaliana (Seq. ID No. 53).~~

Paragraph beginning at line 18 of page 5 has been amended as follows:

Figure 2 - CI-2-like sequences

1. Seq. ID No. 35, Hordeum vulgare (gi:68800)
2. Seq. ID No. 36, Hordeum vulgare (Y08625)
3. Seq. ID No. 37, Zea mays (gi:475922)
4. Seq. ID No. 38, Vicia faba (A21463)
5. Seq. ID No. 39, Cucurbita maxima (S55591, S12897)
6. Seq. ID No. 40, Canavalia lineata (JC2380)
7. Seq. ID No. 41, Vigna angularis (JX0089)
8. Seq. ID No. 42, Nicotiana tabacum (gi:19913)
9. Seq. ID No. 43, Nicotiana glauca (A56555)
10. Seq. ID No. 44, Sambucus nigra (Z46949)
11. Seq. ID No. 45, Momordica charantia (JC2508)
12. Seq. ID No. 46, Cucurbita maxima (S12897)
13. Seq. ID No. 47, Solanum tuberosum (P01052, U30861)
14. Seq. ID No. 48, Solanum tuberosum (U30861)
15. Seq. ID No. 49, Lycopersicon peruvianum (A39547)
16. Seq. ID No. 50, Lycopersicon esculentum (A32067, A24048)
17. Seq. ID No. 51, Lycopersicon esculentum (A24048)
18. Seq. ID No. 52, Amaranthus caudatus (S40496)
19. Seq. ID No. 53, Arabidopsis thaliana (AC005770)
20. Seq. ID No. 33, consensus sequence
22. Seq. ID No. 34, consensus sequence

Paragraphs beginning at line 25 of page 49 through paragraph beginning at line 6 of page 54 have been amended as follows:

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BHL1

The BHL1 insert corresponds to SEQ ID NO 5. Oligonucleotide pairs, N4394/N4395, and N4396/N4397, N4394 (Seq ID NO. 54) /N4395 (Seq ID NO. 55), and N4396 (Seq ID NO. 56)/N4397 (Seq ID NO. 57), were annealed and ligated together to make a 202 base pair double stranded DNA molecule with overhangs compatible with *Rca* I and *Nhe* I restriction sites. PCR was performed on the annealed molecule using primers N5045 (Seq ID NO. 58) and N5046 (Seq ID NO. 59) to add a 5' *Spe* I site and 3' *Hind* III site. The PCR product was then restriction digested at those sites and ligated into pBluescript II-KS+ at *Spe* I and *Hind* III sites. The insert was then removed by restriction digestion with *Rca* I and *Hind* III and was ligated into the *Nco* I and *Hind* III sites of pET28a (Novagen) to form the BHL1 construct.

Oligonucleotide sequences (5' to 3'):

N4394 (Seq ID NO. 54)

1 CATGAAGCTG AAGACAGAGT GGCCGGAGTT GGTGGGGAAA
TCGGTGGAGA

51 AAGCCAAGAA GGTGATCCTG AAGGACAAGC CAGAGGCGCA
AATCATAGTT

101 CTGC

N4395 (Seq ID NO. 55)

1 CAACCGGCAG AACTATGATT TCGCCTCTG GCTTGTCTT
CAGGATCACC

51 TTCTTGCTT TCTCCACCGA TTTCCCCACC AACTCCGGCC
ACTCTGTCTT

101 CAGCTT

N4396 (Seq ID NO. 56)

1 CGGTTGGTAC AAAGGTGACG AAGGAATATA AGATCGACCG
CGTCAAGCTC

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51 TTTGTGGATA AAAAGGACAA CATCGCGCAG GTCCCCAGGG TCGG
N4397 (Seq ID NO. 57)

1 CTAGCCGACC CTGGGGACCT GCGCGATGTT GTCCTTTTAA
TCCACAAAGA

51 GCTTGACGCG GTCGATCTTA TATTCCTTCG TCACCTTTGT AC
N5045 (Seq ID NO. 58)

1 GTACTAGTCA TGAAGCTGAA GACAGA
N5046 (Seq ID NO. 59)

GAGAAGCTTG CTAGCCGACC CTGGGGAC

BHL2

The BHL2 construct insert corresponds to SEQ ID NO 7. An overlap PCR strategy was used to make the BHL2 construct. PWO polymerase from Boehringer-Mannheim was used for all PCR reactions. The primers were chosen to change 3 amino acids in the BHL1 active site loop region, and to create unique Age I and Hind III restriction sites flanking the active site loop, to facilitate loop replacement in future constructs. A unique Rca I site (compatible with Nco I) was included at the 5' end, and a unique Xho I site was included at the 3' end. The overlap PCR was done as follows: PCR was done with primers N13561 (Seq. Id No. 60) and N13564 (Seq. Id No. 63), using the BHL1 construct as template. A separate PCR was done with primers N13563 and ~~13564~~, (Seq. Id No. 62) and N13562 (Seq. Id No. 61) again using the BHL1 construct as template. The products from both reactions were gel purified and combined. Primer N13565 (Seq. Id No. 64), which overlapped regions on both of the PCR products, was then added and another PCR was done to generate the full-length insert. The resulting product was amplified by another PCR with primers N13561 and ~~N13562~~, (Seq. Id No. 60) and N13562 (Seq. Id No. 61). It was subsequently suspected that a deletion was present in N13562 (Seq. Id No. 61) that caused a frameshift near the 3' end of the PCR product. To avoid this frameshift problem, a final PCR reaction was done with primers N13562 (Seq. Id No.

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61) and N13905 (Seq. Id No. 65). The final PCR product was digested with *Rca* I and *Xho* I, and then ligated into the *Nco* I and *Xho* I sites of pET 28b. Note: Some primers had 6-oligonucleotide extensions to improve restriction digestion efficiency. Oligonucleotide sequences (5' TO 3'):

N13561 (Seq. Id No. 60)

1 TTTTTTTCATGAAGCTGAAGACA

N13562 (Seq. Id No. 61) (as ordered)

1 TTTTTTCTCGAGGCTAGCCGACCCTGGGGA

N13563 (Seq. Id No. 62)

1 ATCGACAAGGTCAAGCTTTTTGTGGATAAAAAGGA

N13564 (Seq. Id No. 63)

1 CACCTTTGTACCAACCGGTAGAACTATGATTTGCGC

N13565 (Seq. Id No. 64)

1 GTTGGTACAAAGGTGGCGAAGGCCTATAAGATCGACAAGGTCAAG

N13905 (Seq. Id No. 65)

1 TTTTTTCTCGAGGCTAGCCGACCCTGGGGACCTGCGCTA

BHL3

The BHL3 construct insert corresponds to SEQ ID NO 9. The BHL2 construct was digested with *Age* I and *Hind* III, and the region between these sites was removed by gel purification and discarded. Oligonucleotide pairs, N14471 (Seq. Id No. 66) and N14472 (Seq. Id No. 67), were annealed to make a double stranded DNA molecule with overhangs compatible with *Age* I and *Hind* III restriction sites. The annealed product was ligated into the *Age* I and *Hind* III sites of the digested BHL2 construct to yield the BHL3 construct.

Oligonucleotide sequences (5' to 3'):

N14471 (Seq. Id No. 66)

1 CCGGTTGGTACAAAGGTGGGTAAGCATTATAAGATCGACAAGGTCA

N14472 (Seq. Id No. 67)

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AGCTTGACCTTGTGCGATCTTATAATGCTTACCCACCTTTGTACCAA

BHL3N

The BHL3N construct insert corresponds to SEQ ID No 11. A PCR reaction was done with the BHL3 construct as template. The primers for this reaction were N13771 (Seq. Id No. 68) and N13905 (Seq. Id No. 65). The resulting PCR product was digested with *Rca* I and *Xho* I and ligated into the *Nco* I and *Xho* I sites of pET 28b to yield the BHL3N construct.

Oligonucleotide sequences (5' to 3'):

N13771 (Seq. Id No. 68)

1

TTTTTTCATGAAGTCGGTGGAGAAGAAACCGAAGGGTGTGAAGACAGGTGCG
GGTGACAAGCATAAGCTGAAGACAGAGTG

N13905 (Seq. Id No. 65) (already provided in BHL2 description).

BHL4

The BHL4 construct insert DNA corresponds to SEQ ID NO 13. The BHL2 construct was digested with *Age* I and *Hind* III, and the region between these sites was removed by gel purification and discarded. Oligonucleotide pairs, N22098 (Seq. Id No. 69) and N22099 (Seq. Id No. 70), were annealed to make a double stranded DNA molecule with overhangs compatible with *Age* I and *Hind* III restriction sites. The annealed product was ligated into the *Age* I and *Hind* III sites of the digested BHL2 construct to yield the BHL4 construct.

Oligonucleotide sequences (5' to 3'):

N22098 (Seq. Id No. 69)

CCGGTTGGTACAAAGGTGACGGGCGAATACAAGATCGACCGCGTCA

N22099 (Seq. Id No. 70)

AGCTTGACGCGGTGCGATCTTGTATTGCCCCGTCACCTTTGTACCAA

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BHL5

The BHL5 construct insert DNA corresponds to SEQ ID NO 15. This gene was synthesized by a commercial vendor, The Midland Certified Reagent Company (Midland, Texas). The gene was supplied by Midland following digestion by *Nco* I and *Hind* III, and was ligated into the *Nco* I and *Hind* III sites of pET 28b to yield the BHL5 construct.

BHL6

The BHL6 construct insert DNA corresponds to SEQ ID NO 17. The BHL5 construct was digested with *Age* I and *Sa* I, and the region between these sites was removed by gel purification and discarded. Oligonucleotide pairs, N23923 (Seq. Id No. 71) and N23924 (Seq. Id. No 72), were annealed to make a double stranded DNA molecule with overhangs compatible with *Age* I and *Sa* I restriction sites. The annealed product was ligated into the *Age* I and *Sa* I sites of the digested BHL5 construct to yield the BHL6 construct.

Oligonucleotide sequences (5' to 3'):

N23923 (Seq. Id No. 71)

CCGGTGAATGGAAGATGGATCGCGTCCGCCTCTGGG

N23924 (Seq. Id. No 72)

TCGACCCAGAGGCGGACGCGATCCATCTTCCATTCA

BHL8

The BHL8 construct insert DNA corresponds to SEQ ID No 19. A PCR reaction was done using the BHL6 construct as template. The primers for this reaction were N26671 (Seq ID. No 73) and N26672 (Seq ID. No 74). The resulting PCR product was digested with *Nco* I and *Hind* III and ligated into the *Nco* I and *Hind* III sites of pET 28b to yield the BHL8 construct.

Oligonucleotide sequences (5' to 3'):

N26671 (Seq ID. No 73)

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TTTTTCCATGGCTAAGATGAAGTGCACGTGGCCTGAGCTGGT

N26672 (Seq ID. No 74)

TTTTTAAGCTTGGATCCCTAGCCGCACTTCGGAGTCTTGCGGA